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From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal

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Abstract

Defining the scale of connectivity among marine populations and identifying the barriers to gene flow are tasks of fundamental importance for understanding the genetic structure of populations and for the design of marine reserves. Here, we investigated the population genetic structure at three spatial scales of the red gorgonian *Paramuricea clavata* (Cnidaria, Octocorallia), a key species dwelling in the coralligenous assemblages of the Mediterranean Sea. Colonies of *P. clavata* were collected from 39 locations across the Mediterranean Sea from Morocco to Turkey and analysed using microsatellite loci. Within three regions (Medes, Marseille and North Corsica), sampling was obtained from multiple locations and at different depths. Three different approaches (measures of genetic differentiation, Bayesian clustering and spatially explicit maximum-difference algorithm) were used to determine the pattern of genetic structure. We identified genetic breaks in the spatial distribution of genetic diversity, which were concordant with oceanographic conditions in the Mediterranean Sea. We revealed a high level of genetic differentiation among populations and a pattern of isolation by distance across the studied area and within the three regions, underlining short effective larval dispersal in this species. We observed genetic differentiation among populations in the same locality dwelling at different depths, which may be explained by local oceanographic conditions and which may allow a process of local adaptation of the populations to their environment. We discuss the implications of our results for the conservation of the species, which is exposed to various threats.

Keywords: conservation biology, genetic structure, larval dispersal, mediterranean Sea, microsatellites, *paramuricea clavata*

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Introduction

Dispersal plays a major role in driving marine population connectivity and dynamics, and understanding the level of population connectivity is fundamental to develop suitable conservation strategies and for the design of marine reserves (Shanks *et al.* 2003; Palumbi 2004; Almany *et al.* 2009). However, determining the scale at which dispersal occurs in marine sessile invertebrates is challenging as tracking gametes and larvae in the field is a tough task and barriers to dispersal are much less obvious in marine environment than in terrestrial one (Palumbi 1992, 1994). Molecular markers may be used to distinguish populations, to estimate gene flow among them, to assess demographic parameters and to unveil the signatures of historical and

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current processes that shaped the genetic landscape of populations (Manel *et al.* 2003; Hellberg 2009). Several genetic studies have shown that oceanographic barriers (Baums *et al.* 2006; Patarnello *et al.* 2007; Galarza *et al.* 2009; Sala-Bozano *et al.* 2009) and biological characteristics in marine invertebrates are shaping the genetic structure of the species (Duran *et al.* 2004; Ledoux *et al.* 2010a), suggesting that population connectivity might depend on intrinsic dispersal abilities of the species as well as physical barriers from the environment. Nevertheless, the interplay between life history traits and oceanographic barriers on the genetic structure of the species is not always predictable (Patarnello *et al.* 2007; Galarza *et al.* 2009). Therefore, uncovering population genetic patterns for many species across a shared environment can help to disentangle species-specific effects from environmental effects (Kelly & Palumbi 2010).

In the context of the threats that global changes represent for littoral ecosystems worldwide (IPCC 2007), understanding connectivity for coastal marine species has become a priority for the conservation of these ecosystems. A major effort has been devoted to assess connectivity in a wide variety of taxa in coral reef ecosystems (Van Oppen & Gates 2006; Jones *et al.* 2009), because of the documented decline in coral cover in recent decades (Gardner *et al.* 2003; Bruno & Selig 2007) related to local and global stressors (Hughes *et al.* 2003). During the last decade, the coralligenous assemblages of the Mediterranean Sea, whose main structural components are low-turnover organisms (Coma *et al.* 1998; Garrabou *et al.* 2002), have also suffered from various local and global threats such as pollution, fishing, diving, invasive species and mass mortality events possibly linked to climate change (Ballesteros 2006). However, despite these threats and the need to extend our knowledge of population dynamics and connectivity for conservation and ecosystem-based management purposes (Gaines *et al.* 2010), genetic studies on species from the coralligenous assemblages are still scarce (see Abbiati *et al.* 2009 for a review). Previous genetic studies revealed strong population genetic structure and limited larval dispersal for some coralligenous species such as the red coral *Corallium rubrum* (Costantini *et al.* 2007a; b; Ledoux *et al.* 2010b) or the sponge *Crambe crambe* (Duran *et al.* 2004), even at small scales (Ledoux *et al.* 2010b, Calderón *et al.* 2007; Ledoux *et al.* 2010a; respectively). However, these studies did not always simultaneously include different spatial scales (but see Ledoux *et al.* 2010b), which can provide more insight into the importance of various factors in shaping genetic patterns.

The red gorgonian, *Paramuricea clavata* (Risso, 1826), is a colonial and sessile marine invertebrate inhabiting the Mediterranean coralligenous assemblages. This species is a long-lived (50–100 years) and slow-growing gorgonian (Coma *et al.* 1998). *Paramuricea clavata* acts as an ecosystem engineer, playing an important role in the structural complexity and maintenance of biomass and biodiversity in the associated assemblages (True 1970; Ballesteros 2006). It is widely distributed in the western part of the Mediterranean Sea and in the Adriatic Sea (Carpine & Grasshof 1975) where it exhibits a discontinuous distribution. Red gorgonians are less commonly found in the Aegean Sea (Öztürk *et al.* 2004) and in the neighbouring Atlantic Ocean (J. G Harmelin, personal communication). The bathymetric distribution of the species extends from 5 m to beyond 200 m (P. Chevaldonné & T. Pérez, personal communication). However, the species exhibits a decrease in depth of its upper limit distribution along the latitudinal gradient (Linares *et al.* 2008a). *Paramuricea clavata* is a gonochoric surface brooder species (Coma *et al.* 1995). Once released, the planula larvae display negative phototaxis behaviour (Linares *et al.* 2008b) and remain in suspension for a few minutes before dropping to the bottom (Coma *et al.* 1995). However, the larval phase can span between 6 and 23 days under experimental conditions (Linares *et al.* 2008b). The assemblages shaped by red gorgonians constitute one of the most attractive seascapes in the Mediterranean Sea (Harmelin & Marinopoulos 1994). However, this species is affected by the previously mentioned threats to coralligenous assemblages, including mass mortality events (Cerrano *et al.* 2000; Linares *et al.* 2005; Garrabou *et al.* 2009), which have serious consequences at the population and colony levels (Linares *et al.* 2005, 2008c).

The aim of this study was to unveil the genetic structure of a sessile and long-lived species, *Paramuricea clavata*, across the Mediterranean Sea, at three spatial scales: global (3000 km), regional (from 200 to 52 km) and local (over depth ranges, from 10 to 40 m). More specifically, we (i) assessed the genetic diversity and population structure of this species over its distribution range (global scale), (ii) evaluated connectivity among populations within three regions (regional scale) and at different depths within the same locality (local scale) and (iii) identified putative barriers to gene flow. Finally, we discuss the implications of our results for the conservation of *P. clavata* and other species of the coralligenous assemblages, one of the most biodiversity-rich communities in the Mediterranean Sea.

Materials and methods

Sampling design

Paramuricea clavata colonies were randomly collected by SCUBA diving following a hierarchical sampling design.

Thirty-nine locations or depths were sampled within the Mediterranean Sea to cover most of the distribution range of the species and to allow the study of genetic structure at distances varying from 10 m to 3000 km (Fig. 1; Table 1). Three regions (Medes, Marseille and North Corsica) were sampled at multiple locations, and five locations (Pota del Llop, Pharillons, Grotte Pérès, Petit Congloué and Riou Sud) were sampled at two different depths (Table 1). For each site, about 30 colonies of approximately the same height (approximately 15 cm) were sampled and a small fragment preserved in 95% ethanol at −20 °C prior to extraction.

Microsatellite genotyping and polymorphism

Total genomic DNA was extracted from 1114 individuals using a salting out procedure adapted from Miller *et al.* (1988) (see Supporting Information for

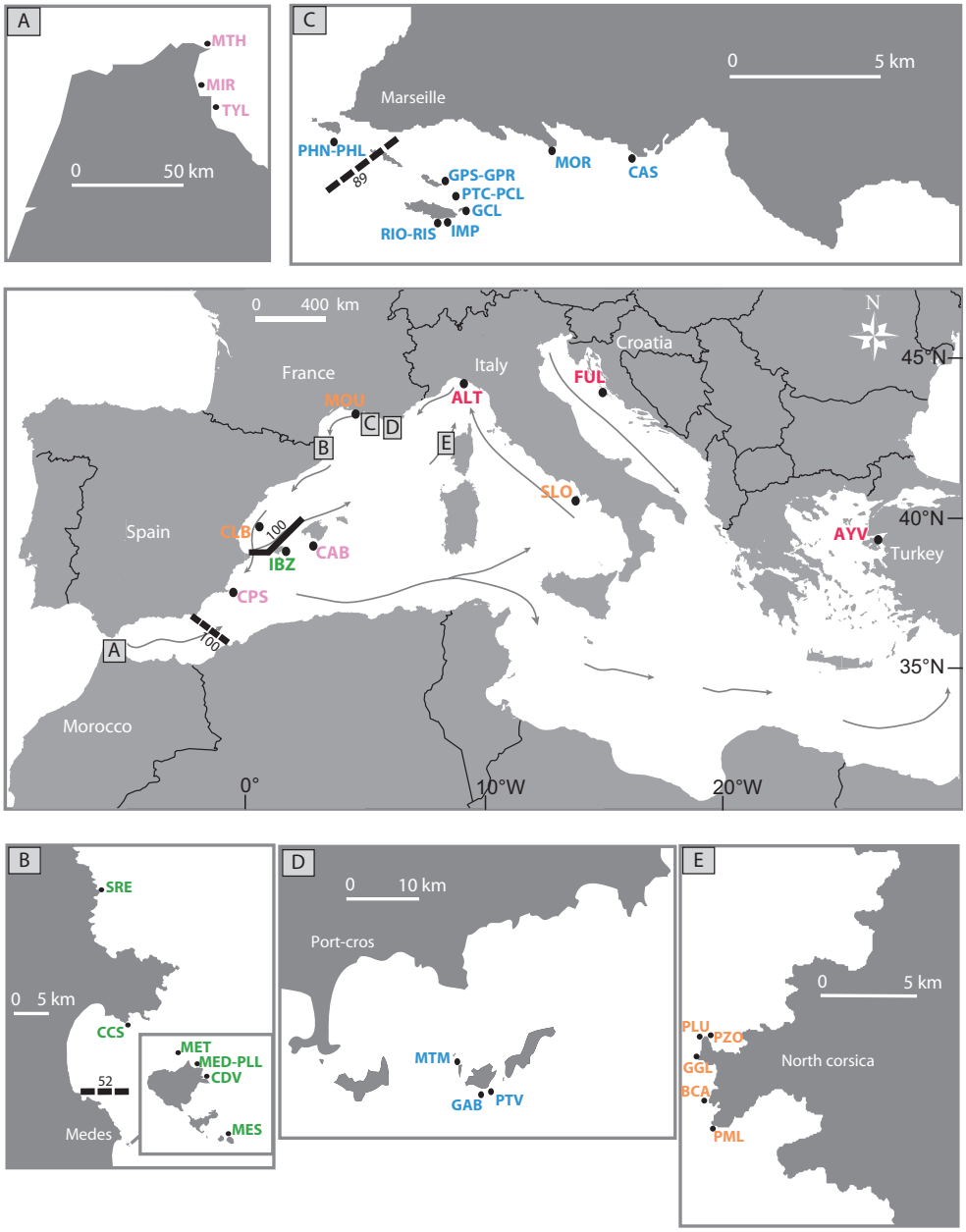

Fig. 1 Map of the 39 *Paramuricea clavata* samples (black dots). Samples collected at two different depths in the same location are separated by a hyphen. The colours of the sample names correspond to their assignment to STRUCTURE clusters for $K = 5$. Details of samples are given in Table 1. The dominant sea surface currents according to Pinardi & Masetti (2000) are indicated by grey arrows. Black solid and black-dotted lines represent the location of the main genetic breaks as revealed by BARRIER during the analysis at Mediterranean scale and the within cluster analysis, respectively (the associated bootstrap values are given).

Table 1 Location of *Paramuricea clavata* samples

Basin	Location name	Population label	GPS coordinates		Depth (m)	Sample size
			Latitude	Longitude		
Alboran Sea	Monte Hacho (Ceuta, Spain)	MTH	35°55′5.78″N	5°17′59.27″O	26	27
Alboran Sea	Marinasmir (Morocco)	MIR	35°45′6.00″N	5°18′48.00″O	40	27
Alboran Sea	Martyl (Morocco)	TYL	35°38′30.00″N	5°14′54.00″O	35	27
Alboran Sea	Bajo de Fuera (Cap de Palos, Spain)	CPS	37°39′8.64″N	0°39′8.62″O	40	28
Balearic Sea	Bledes (Na Gorra) (Ibiza Island, Spain)	IBZ	38°58′13.21″N	1°40′11.93″E	37	30
Balearic Sea	L'Imperial (Cabrera Island, Spain)	CAB	39°7′25.40″N	2°57′37.22″E	40	19
Balearic Sea	Bajo del Carallot (Columbretes Island, Spain)	CLB	39°53′32.40″N	0°40′13.55″E	40	26
Catalan Sea	Carall Bernat (Medes Islands, Spain)	MES	42°2′31.92″N	3°13′41.38″E	20	24
Catalan Sea	Cova de la Vaca (Medes Islands, Spain)	CDV	42°2′52.97″N	3°13′34.76″E	17	36
Catalan Sea	Pota del Llop (Medes Islands, Spain)	MED	42°2′58.92″N	3°13′31.44″E	15	27
Catalan Sea	Pota del llop (Medes Islands, Spain)	PLL	42°2′58.92″N	3°13′31.44″E	35	27
Catalan Sea	Medallot (Medes Islands, Spain)	MET	42°3′5.62″N	3°13′18.31″E	20	27
Catalan Sea	Punta Falconera (Cap de Creus)	CCS	42°13′56.89″N	3°13′7.95″E	25	27
Catalan Sea	Sec Rederis (Banyuls, France)	SRE	42°27′50.76″N	3°9′59.69″E	20	27
Gulf of Lion	Tombant Moulon Est (Cote Bleue, France)	MOU	43°19′51.00″N	5°14′22.00″E	20	27
Gulf of Lion	Pharillons (Marseille, France)	PHN	43°12′26.64″N	5°20′17.52″E	20	27
Gulf of Lion	Pharillons (Marseille, France)	PHL	43°12′26.64″N	5°20′17.52″E	40	28
Gulf of Lion	Grotte Peres (Marseille, France)	GPS	43°11′11.62″N	5°23′28.16″E	10	29
Gulf of Lion	Grotte Peres (Marseille, France)	GPR	43°11′11.62″N	5°23′28.16″E	20	38
Gulf of Lion	Petit Congloue (Marseille, France)	PTC	43°10′45.33″N	5°23′43.92″E	10	33
Gulf of Lion	Petit Congloue (Marseille, France)	PCL	43°10′45.33″N	5°23′43.92″E	20	27
Gulf of Lion	Grand Congloue (Marseille, France)	GCL	43°10′32.79″N	5°24′6.62″E	7	35
Gulf of Lion	Imperiales de Terre (Marseille, France)	IMP	43°10′22.79″N	5°23′35.39″E	8	33
Gulf of Lion	Riou Sud (Marseille, France)	RIO	43°10′21.66″N	5°23′25.16″E	20	31
Gulf of Lion	Riou Sud (Marseille, France)	RIS	43°10′21.66″N	5°23′25.16″E	40	26
Gulf of Lion	Morgiou (Marseille, France)	MOR	43°12′3.36″N	5°27′5.22″E	30	31
Gulf of Lion	Castelvieil (Marseille, France)	CAS	43°11′51.36″N	5°29′55.50″E	10	30
Ligurian Sea	Montremian (Port-Cros, France)	MTM	43°1 ′7.15″N	6°21′45.98″E	20	29
Ligurian Sea	Gabiniere (Port-Cros, France)	GAB	42°59′21.54″N	6°23′49.18″E	22	32
Ligurian Sea	Pointe du Vaisseau (Port-Cros, France)	PTV	42°59′42.92″N	6°24′24.17″E	20	29
Ligurian Sea	Palazzinu (North Corsica, France)	PZO	42°22′46.69″N	8°32′58.13″E	25	27
Ligurian Sea	Palazzu (North Corsica, France)	PLU	42°22′48.65″N	8°32′46.85″E	28	27
Ligurian Sea	Garganellu (North Corsica, France)	GGL	42°22′21.36″N	8°32′12.84″E	20	27
Ligurian Sea	Baja Casju (North Corsica, France)	BCA	42°20′58.56″N	8°33′3.42″E	25	27
Ligurian Sea	Punta Muchillina (North Corsica, France)	PML	42°19′55.92″N	8°33′12.51″E	20	27
Ligurian Sea	Altare (Portofino, Italy)	ALT	44°18′20.40″N	9°11′45.90″E	25	36
Tyrrhenian Sea	Sant'Angelo (Ischia Island, Italy)	SLO	40°41′30.98″N	13°53′36.69″E	32	27
Adriatic Sea	Fulija Island (dugi otok) (Croatia)	FUL	44°1′5.52″N	15°6′39.96″E	40	31
Aegean Sea	Ayvalik (Ezerbey Sigiligi) (Turkey)	AYV	39°22′12.23″N	26°34′36.21″E	35	21

details). All individuals were genotyped at six microsatellite loci: Parcla 09, Parcla 10, Parcla 12, Parcla 14, Parcla 17 (Molecular Ecology Resources Primer Development Consortium *et al.* 2010) and Par_d (Agell *et al.* 2009). All the loci were amplified according to the PCR protocol described in Molecular Ecology Resources Primer Development Consortium *et al.* (2010). PCR products were analysed on an ABI 3130 Genetic Analyser using an internal size standard (GeneScan 600 LIZ; Applied Biosystems). GeneMapper v.3.5 software (Applied Biosystems) was used to score alleles.

We used MICRO-CHECKER v.2.2.3 (Van Oosterhout *et al.* 2004) to check for scoring errors owing to stutters, large allele dropout and to estimate null allele frequencies.

Linkage disequilibrium was tested among all pairs of loci in each sample with a permutation test ($n = 1000$) using GENETIX v.4.05 (Belkhir *et al.* 2004). Tests for Hardy–Weinberg equilibrium within sample for each locus and over all loci were conducted with GENEPOP v.4.0 (Rousset 2008). The level of significance was determined by a Markov chain method (Guo & Thompson 1992) using the default parameters. Single

and multilocus Weir & Cockerham's (1984) f estimator of F_{IS} were computed with GENEPOP.

We analysed the genetic diversity for each sample by computing observed (Ho) and Nei's (1973) unbiased expected heterozygosity (He) with GENETIX. Allelic richness [Ar(g)] and private allelic richness [Ap(g)] were estimated with a rarefaction procedure using the HP-RARE software (Kalinowski 2005) with the minimum number of genes set to 18.

Analysis of genetic structure at different spatial scales and estimation of dispersal

We investigated population structure using different approaches: measures of genetic differentiation (F_{ST}, D_{EST}), a Bayesian clustering method, a hierarchical analysis of molecular variance and a spatially explicit maximum-difference algorithm. For the measures of genetic differentiation, samples were used as population unit. We computed Weir & Cockerham's (1984) θ estimator of F_{ST} with GENEPOP. As null alleles can induce overestimation of genetic distance (Chapuis & Estoup 2007), pairwise F_{ST} estimates were also computed following the excluding null allele (ENA) method in FREENA (Chapuis & Estoup 2007). Jost's (2008) measure of genetic differentiation (D_{EST}) was computed with SMOGD v.1.2.5 (Crawford 2010). We tested the significance of pairwise genotypic differentiation between samples with an exact test as implemented in GENEPOP using the default parameters.

The pattern of isolation by distance (IBD) at Mediterranean scale and within the three regions was tested through the correlation between pairwise $F_{ST}/(1-F_{ST})$ values and the logarithm of the geographical distances between populations by a Mantel test (n = 10 000 randomizations; Rousset 1997) with IBDWS 3.16 (Jensen *et al.* 2005).

The Bayesian method implemented in STRUCTURE v.2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007) was used to evaluate the number of clusters (K) in our data set from individual's genotypes without prior information on their geographical locations. STRUCTURE was launched under the admixture model, with correlated allele frequencies among clusters and the recessive allele option to cope with null alleles (Falush *et al.* 2007). Ten independent runs were performed for each K using 500 000 iterations and a burn-in period of 50 000. As suggested by Rosenberg *et al.* (2001, 2002) and Jakobsson *et al.* (2008) for cases of large data sets, we adopted a hierarchical approach. As detection of population structure at fine-scale levels is indeed limited when the data set is large, this approach thus allows for a more complete description of the patterns of structure (see for example Garnier *et al.* 2004; Wang *et al.* 2007; Ledoux *et al.* 2010b). We first ran STRUCTURE on the whole data set (i.e. Mediterranean scale) with K varying from 1 to 15. Because no single K value provided a full description of population structure because of the additional substructure (Jakobsson *et al.* 2008), we discussed the values between 2 and 5. The clustering solution observed for K = 5 was then retained to subdivide the samples into five data sets for a second round of STRUCTURE for the study at regional scale (with K varying from 1 to the maximum number of samples within each cluster). Given the current debate on how to estimate the number of clusters (Pritchard *et al.* 2000; Evanno *et al.* 2005; Waples & Gaggiotti 2006; François & Durand 2010), we opted for the 'standard and conservative' approach of Pritchard *et al.* (2007) to select a K value for this second round of clustering. We plotted the log probability of the data (LnP(D)) as a function of K across the 10 runs and looked for the value that captured the major structure in the data (Pritchard *et al.* 2007). CLUMPP v.1.1 (Jakobsson & Rosenberg 2007) was used to merge the results across the 10 runs for a given K when a single clustering solution was found; otherwise, only runs with the same mode (symmetric similarity coefficient >0.9) were merged, and the most frequently occurring mode was retained. DISTRUCT v.1.1 (Rosenberg 2004) was used to visualize the results.

ARLEQUIN v.3.5 (Excoffier *et al.* 2005) was used to perform hierarchical analysis of molecular variance (AMOVA, n = 1000 permutations) using the groups defined by STRUCTURE for K = 5 (see Results).

We ran the software BARRIER v. 2.2 (Manni *et al.* 2004) to identify barriers to gene flow represented by zones of abrupt change in the pattern of genetic variation on the whole data set (i.e. Mediterranean scale) and within each STRUCTURE cluster (i.e. regional scale). The software computes barriers on a Delaunay triangulation (built with GPS coordinates) using Monmonier's (1973) maximum-difference algorithm (with pairwise F_{ST} matrix). The robustness of the identified barriers was tested with 100 resampled bootstrap matrices created with an R function (Eric Petit, UMR ECOBIO CNRS, Paimpont, personal communication).

We used a Bayesian assignment method (Rannala & Mountain 1997) as implemented in GENECLASS2 (Piry *et al.* 2004) to identify putative first-generation migrants among populations. A Monte Carlo resampling method was performed to compute individual probability of assignment to each population (Paetkau *et al.* 2004) using 10 000 simulated individuals.

We computed with SPAGEDI (Hardy & Vekemans 2002) the relationship coefficient Moran's I (1948) among pairwise individuals belonging to the same location and depth and among pairwise individuals belong-

ing to the same location but to different depths. For each location, statistical differences between mean Moran's I values among individuals from the same location and depth and mean Moran's I value among individuals from the same location but different depths were investigated with a Kruskal–Wallis ANOVA.

Whenever multiple tests were conducted, the level of significance was adjusted using a false discovery rate (FDR) (Benjamini & Hochberg 1995).

Results

Genetic variability

No evidence of large allele dropout or scoring errors owing to stutters was found using MICRO-CHECKER. No global significant linkage disequilibrium among loci was found ($P > 0.05$ after FDR correction) on the overall samples. All the loci were polymorphic with total number of alleles ranging from 10 for Par_d to 35 for Parcla 09 and Parcla 12 and with a mean value of 25 alleles per locus. Observed and unbiased expected heterozygosities ranged from 0.58 for TYL to 0.83 for PZO and from 0.56 for AYV to 0.81 for PHL, respectively (with a mean value of 0.69 and 0.74, respectively) (Table 2). The allelic richness Ar(18) varied from 4.41 (FUL) to 7.55 (PHL). Significant differences in allelic richness were observed between the five clusters defined by STRUCTURE (Kruskal–Wallis, $P = 0.001$) with higher values found in clusters 2, 3 and 4 (7.0, 6.61 and 6.30, respectively) and lower values in clusters 5 and 1 (4.93 and 5.93, respectively). The private allelic richness Ap(18) ranged from 0 (CLB, CCS, PHN, GCL, CAS, PTV, PLU, PML) to 0.72 (TYL), and no differences were observed when comparing the five clusters ($P = 0.097$). Multilocus F_{IS} values ranged between −0.11 for AYV and 0.23 for TYL (Table 2). Over all loci, significant heterozygote deficiencies were found in 23 samples of 39 (after FDR correction). Examining each locus separately, F_{IS} values ranged from −0.448 for Parcla 09 in AYV sample to 0.705 for Parcla 12 in MTH (Table S1, Supporting Information). Heterozygote deficiency was not generalized for all loci in all samples. In case of departure from Hardy–Weinberg equilibrium, evidence for null allele was checked and null allele frequencies were computed at each locus in each sample. The estimates of null allele frequencies varied between 0.07 for Parcla 09 in MED sample and 0.28 for Parcla 12 in MIR sample, with a mean value of 0.14 over all loci and samples (Table S1, Supporting Information). However, in some cases, the F_{IS} value was significant but no evidence of null allele was detected according to MICRO-CHECKER (Table S1, Supporting Information).

Table 2 Estimators of genetic diversity in 39 samples of *P. clavata* at six microsatellite loci

Population	Ho	He	Na	Ar(18)	Ap(18)	*f*
MTH	0.62	0.78	9.67	6.45	0.1	**0.21**
MIR	0.64	0.75	8.33	5.98	0.28	**0.11**
TYL	0.58	0.77	10.17	6.56	0.72	**0.23**
CPS	0.62	0.73	7.5	5.58	0.01	**0.15**
IBZ	0.65	0.66	8.67	5.44	0.12	**0.01**
CAB	0.69	0.67	6.17	5.07	0.29	−0.02
CLB	0.66	0.77	8	6.03	0	**0.17**
MES	0.72	0.73	9.33	6.88	0.01	0.01
CDV	0.69	0.73	10.33	6.59	0.08	**0.06**
MED	0.75	0.78	10.33	7.11	0.06	0.04
PLL	0.71	0.75	11.5	7.43	0.13	0.05
MET	0.63	0.71	10.17	6.99	0.06	**0.11**
CCS	0.69	0.75	9.67	6.54	0	0.08
SRE	0.6	0.7	9.17	5.9	0.04	0.11
MOU	0.75	0.77	8	6.12	0.02	**0.02**
PHN	0.68	0.74	8.33	6.58	0	**0.1**
PHL	0.75	0.81	11.5	7.55	0.13	**0.07**
GPS	0.68	0.72	10.5	6.7	0.01	0.07
GPR	0.76	0.77	12.5	7.44	0.16	0.02
PTC	0.73	0.8	11	7.35	0.02	**0.08**
PCL	0.74	0.77	10.5	7.44	0.01	0.04
GCL	0.77	0.8	9.33	6.92	0	**0.03**
IMP	0.71	0.75	12.33	7.47	0.11	**0.06**
RIO	0.65	0.76	11.67	7.52	0.02	**0.16**
RIS	0.7	0.75	11	7.05	0.01	**0.07**
MOR	0.72	0.76	10.67	7.23	0.01	**0.05**
CAS	0.61	0.71	8.67	6.38	0	**0.14**
MTM	0.63	0.68	8.17	5.66	0.04	0.07
GAB	0.7	0.76	10.67	7.24	0.05	**0.07**
PTV	0.66	0.67	9.83	6.46	0	**0.03**
PZO	0.83	0.78	10.17	6.95	0.07	−0.07
PLU	0.8	0.78	8.17	6.32	0	−0.01
GGL	0.7	0.76	9.17	6.67	0.08	**0.06**
BCA	0.73	0.78	8.33	6.1	0.09	0.07
PML	0.68	0.71	7.5	5.59	0	0.04
ALT	0.66	0.69	8.5	5.76	0.04	0.04
SLO	0.67	0.77	9	6.59	0.19	**0.13**
FUL	0.63	0.66	6	4.41	0.06	**0.04**
AYV	0.61	0.56	6.17	4.63	0.13	−0.11
Mean Value	0.69	0.74	9.4	6.48	0.08	**0.07**

Ho, observed heterozygosity; He, unbiased expected heterozygosity; Na, number of alleles; Ar(18) and Ap(18), rarefied allelic and private allelic richness, respectively (with rarefaction size of 18); *f*, Weir & Cockerham's (1984) *f* estimator of F_{IS} with significant values in bold (0.05 threshold after FDR correction).

Population genetic structure

Overall F_{ST} value was strong (0.116), and the exact test indicated a highly significant differentiation. Pairwise F_{ST} values (Table S2, Supporting Information) ranged from 0.004 for PLL vs. MET to 0.330 for IBZ vs. AVY. No significant differences were observed between

pairwise F_{ST} and pairwise F_{ST} corrected for null alleles (*t*-test, $P = 0.219$), suggesting that null alleles did not affect this analysis. Pairwise D_{EST} values (Table S2, Supporting Information) ranged from 0.001 for MET vs. PLL to 0.738 for SLO vs. TYL with a mean value of 0.319. All pairwise differentiation tests were significant except 6 of 741 (after FDR correction). The nonsignificant comparisons included samples from Medes separated by distances of 920 and 370 m (MES vs. PLL and PLL vs. MET, respectively); samples from Marseille separated by 1220 m (PCL vs. IMP); and samples belonging to the same location but to different depths from Medes and Marseille (MED vs. PLL; PTC vs. PCL; and GPS vs. GPR separated by 20, 10 and 10 m, respectively). Significant genetic differentiation was observed between some samples collected at different depths and separated by 20 m (PHN vs. PHL and RIO vs. RIS).

Positive and significant correlation between $F_{ST}/(1-F_{ST})$ values and the logarithm of the geographical distances was found ($R^2 = 0.509$, $P < 0.0001$), suggesting a strong pattern of IBD at Mediterranean scale (Fig. 2). Within the three regions, Medes, Marseille and North Corsica, a pattern of IBD was also evidenced ($R^2 = 0.584$, $P = 0.026$; $R^2 = 0.080$, $P = 0.031$; and $R^2 = 0.264$, $P = 0.046$, respectively) (Fig. S1, Supporting Information). Similar patterns of IBD were observed with D_{EST} (data not shown).

For the first round of STRUCTURE, the plot of LnP(D) as a function of K did not reveal a clear plateau but a weak increase in LnP(D) values from $K = 1$ to 15 (Fig. S2, Supporting Information). For $K = 2$, the main separation appeared between samples from the Alboran Sea, the Balearic Sea (CAB, CLB) and the Ligurian Sea (North Corsica) (cluster 1) and samples from the Gulf of Lion (Marseille) and the Ligurian Sea (Port-Cros and Portofino) (cluster 2) (Fig. 3). All other samples were more or less equally assigned to each of the two clusters. For $K = 3$, samples from the Catalan Sea, one sample from the Balearic Sea (IBZ) and samples from the Ligurian Sea (North Corsica, except PLU) were grouped in a third cluster. For $K = 4$, samples from the Ligurian Sea (North Corsica) (cluster 4) were clearly distinguished from samples from the Catalan Sea. At $K = 5$, a fifth cluster appeared with one sample from the Ligurian Sea (Portofino, ALT) and the samples from the Adriatic and Aegean Seas (FUL and AYV, respectively). Overall, the five clusters were congruent with the geographical origin of the samples, with a few exceptions: IBZ, CAB, CLB from the Balearic Sea were not grouped together; MOU from Côte Bleue (Gulf of Lion) and SLO from the Tyrrhenian Sea were clustered with North Corsican samples (Ligurian Sea); and ALT from the Ligurian Sea was gathered with FUL and AYV from eastern Mediterranean Sea (Adriatic and Aegean Seas, respectively). Each of the five clusters was analysed for a second round. During the second round, the value that captured the major structure in the data was retained. For cluster 1, K was set to 3 with the three samples from the Moroccan coast grouping together and CPS and CAB representing each a cluster. For cluster 2, K was set to 3. Samples from Port-Cros (MTM, GAB and PTV) and MOR belonged to one cluster, another grouped PHN, PHL and RIS and the third GPS and GPR, whereas the other samples from Marseille showed a high proportion of admixture of these two last clusters. For cluster 3, $K = 3$ was the retained solution with one cluster regrouping all the samples from Medes and IBZ and CCS/SRE representing each a cluster. For cluster 4, the retained solution was $K = 4$. The samples from North Corsica were divided into 2 clusters: [PZO, PLU, PML] and [GGL, BCA] with PML displaying a high coefficient of population membership and the other samples exhibiting a high level of admixture. For cluster 5, the retained solution was $K = 3$ with ALT, FUL and AYV representing their own cluster.

The AMOVA revealed a highly significant genetic structuring among the STRUCTURE groups, among samples within groups and within samples ($P < 0.001$; Table 3). The percentage of genetic variation explained by differences among groups was almost equal to the percentage of genetic variation explained by differences among samples within groups (6.0% and 7.2%, respectively),

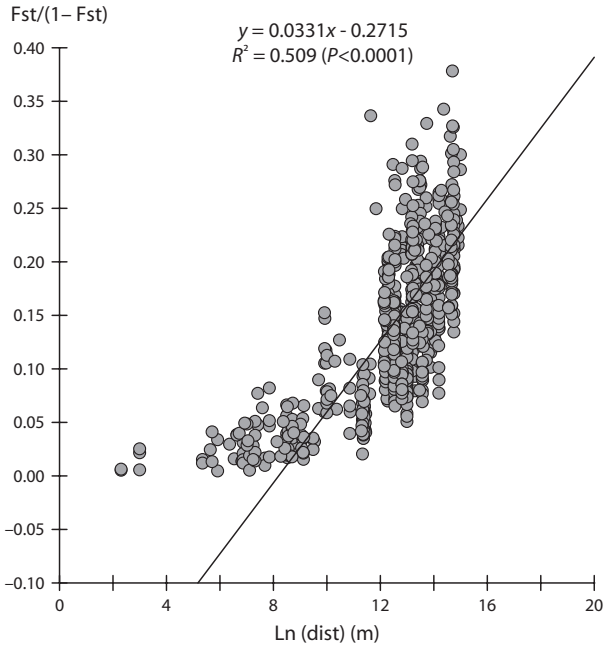

Fig. 2 Correlation between genetic distances computed as $F_{ST}/(1-F_{ST})$ and the logarithm of geographical distances (m) between sample pairs.

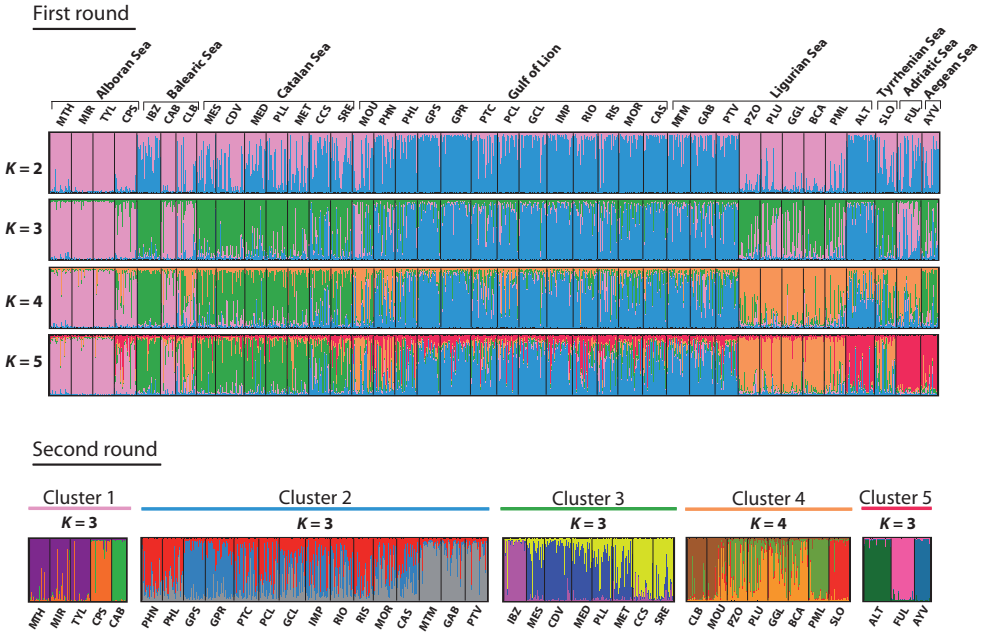

Fig. 3 Bar plot from the Bayesian clustering analysis with STRUCTURE, revealing population structure of *P. clavata* during the first round (first five panels) and second round (last panel).

Table 3 Analysis of molecular variance (AMOVA) of *P. clavata* samples grouped according to STRUCTURE clusters

Source of variation	d.f.	% of variance	*P*-value
STRUCTURE clusters $K = 5$			
Among groups	4	6.0	<0.001
Among samples within group	34	7.2	<0.001
Within samples	2105	86.8	<0.001

and the highest percentage of genetic variation was found within samples (86.8%) (Table 3).

The software BARRIER revealed the occurrence of sharp breaks in genetic differentiation (barriers) across the studied area. However, given our geographical sampling scheme, we have considered only the most relevant barrier that was located for the global analysis between the Balearic Islands and the Spanish coast and between the northern and southern Spanish coast around the Ibiza channel (Fig. 1, solid line). This barrier was supported by a bootstrap value of 100. We ran BARRIER a second time inside each STRUCTURE cluster by simulating one barrier (Fig. 1, dotted lines). For cluster 1, the barrier appeared between MTH/MIR/TYL and CPS/CAB (bootstrap value of 100). Within cluster 2, the break was located between the 2 western samples of Marseille and the remaining samples (bootstrap value of 89). For cluster 3, the barrier was placed between the samples from Medes and CCS/SRE but had a low bootstrap value (52% bootstrap). Within cluster 4, the barrier separated CLB/MOU from all other samples (87% bootstrap). For cluster 5, the genetic break was located between ALT and the samples from the eastern basin (100% bootstrap). These two last barriers were not represented in Fig. 1 as they involved samples situated far apart and they could not be unambiguously located.

Populations exchanged very few migrants, according to GENECLASS2, with only 3.9% of the individuals detected as migrants (Table S3, Supporting Information). The origin of almost all detected migrants was consistent with predominant surface currents, and the mean distance travelled from their population of origin was estimated at 160 km.

At the local scale, we aimed to assess the effect of depth on genetic structuring. To evaluate the genetic similarity between individuals collected in the same location, the mean pairwise Moran's I values among individuals from the same location and depth and among individuals from the same location but different depths were computed (Table 4). For three locations (Pota del Llop, Grotte Pérès and Petit Congloué), the mean pairwise Moran's I values among individuals from the same depth were low and not significantly different from the mean pairwise Moran's I value among individuals separated by depth. For the two locations with samples at 20 and 40 m (Pharillons and Riou Sud), the mean pairwise Moran's I values among individuals from the same depth were high and significantly higher than the mean pairwise Moran's I value among individuals separated by depth.

Table 4 Mean pairwise Moran's I values among individuals within location and between depths. Statistical differences are assessed by a Kruskal–Wallis ANOVA and the corresponding *P*-values reported

	Pota del Llop		Grotte Peres		Petit Congloue		Pharillons		Riou Sud	
Location	MED (15 m)	PLL (35 m)	GPS (10 m)	GPR (18 m)	PTC (10 m)	PCL (20 m)	PHN (20 m)	PHL (40 m)	RIO (20 m)	RIS (40 m)
Mean pairwise	0.005 ± 0.191	0.006 ± 0.152	0.007 ± 0.206	0.004 ± 0.158	0.003 ± 0.173	0.009 ± 0.190	0.019 ± 0.220	0.013 ± 0.193	0.018 ± 0.170	0.029 ± 0.174
Individual Moran's I	−0.005 ± 0.170		−0.005 ± 0.168		−0.005 ± 0.168		−0.016 ± 0.177		−0.022 ± 0.153	
P	0.301		0.474		0.883		0.022		0.000	

Discussion

The analysis of six microsatellite loci evidenced strong genetic differentiation among populations of *Paramuricea clavata* separated by distances ranging from thousands kilometres to twenty metres. A strong pattern of isolation by distance was observed at the global as well as the regional scales. At the local scale, depth differentiation was also found in some locations. At the sample level, we observed a high level of genetic diversity and heterozygote deficiency. Many populations of sessile marine invertebrates from different taxa, displaying restricted larval dispersal, exhibit departure from Hardy–Weinberg equilibrium because of heterozygote deficiency, such as sponges (Duran *et al.* 2004), corals (Polato *et al.* 2010), ascidians (Pérez-Portela & Turon 2008; Dupont *et al.* 2009) and gorgonians (Gutiérrez-Rodríguez & Lasker 2004; Ledoux *et al.* 2010a,b). Several factors may contribute to the observed heterozygote deficiencies, such as null alleles, inbreeding and temporal and spatial Wahlund effects (Addison & Hart 2005). Although we detected null alleles, they were not generalized in all samples for which we observed heterozygote deficiency. Thus, biological factors may also contribute to explain the observed heterozygote deficiencies. The reproductive biology of this species may lead to inbreeding because: (i) females brood the eggs on their surface and thus the fertilization relies on sperm dispersal which, in other gorgonian species, has been documented to decrease at the scale of a few metres (Coma & Lasker 1997a,b) and (ii) after release, many of the larvae of *Paramuricea clavata* settle near the putative maternal colony (Coma *et al.* 1995; Linares *et al.* 2008b). Combination of these two processes increases the probability of mating between related individuals. Inbreeding is a likely cause, yet spatial Wahlund effect could not be refuted as a possible explanation.

Genetic structure at three spatial scales

At the Mediterranean scale, *Paramuricea clavata* showed, over all loci, high and highly significant genetic structure. The first round of the Bayesian clustering method revealed discontinuities in the spatial distribution of genetic diversity congruent with the geographical locations of the samples (Fig. 3). A clear distinction between southern, northern–western and eastern samples within the Mediterranean Sea was revealed. In the north-western Mediterranean Sea, three clusters were, respectively, represented by samples from Medes (Catalan Sea), Marseille (Gulf of Lion) and North Corsica (Ligurian Sea) and tally with the genetic structure of *Corallium rubrum* within this region, which suggests that similar mechanisms may act on the genetic structure

of these two species in the NW Mediterranean Sea (Ledoux *et al.* 2010b).

Hydrodynamic processes and oceanographic barriers in the Mediterranean Sea may contribute to the genetic structure of *P. clavata*. The strongest barrier identified may correspond to the joint effect of the Balearic front and the Ibiza channel. The Balearic front is a shelf/-slope front produced by thermohaline differences between three surface water masses of the Balearic Sea and characterized by its mesoscale variability (García *et al.* 1994). This front has been reported to act as a barrier to gene flow between the Balearic Islands and the Spanish coast opposite, for several fish species whatever their early-life history traits (Galarza *et al.* 2009). The Balearic Sea, by its central position between the Liguro-Provençal and the Alboran/Algerian basins, plays an important role in the water circulation of the western Mediterranean Sea (García *et al.* 1994). The Mediterranean surface and intermediate waters encounter the less saline Atlantic waters at the Ibiza channel (Fernández *et al.* 2005). During spring and early summer, the formation of an anticyclonic gyre causes the deviation of the northern current to the Balearic Islands (Monserrat *et al.* 2008) impeding the water flow from crossing the Ibiza channel and to reaching the southern Spanish coast. *Paramuricea clavata* reproduces between June and July; thus, the southward larval transport along the Spanish coast may be hindered at the Ibiza channel by this gyre. The effect of the Ibiza channel reducing gene flow has been emphasized for the comber *Serranus cabrilla* (Schunter *et al.* unpublished) and the decapod crustacean *Liocarcinus depurator* (Garcia-Merchan *et al.* unpublished). Our results showed that the Balearic Sea is composed of a mix of gene pools from different origins: Catalan Sea (IBZ), Alboran Sea (CAB) and Ligurian Sea (CLB), which may be due to oceanographic conditions in this region. Several studies have highlighted the peculiar status of the Balearic region for the red coral *Corallium rubrum* (Ledoux *et al.* 2010b), the seagrass *Posidonia oceanica* (Rozenfeld *et al.* 2008) and the comber *Serranus cabrilla* (Schunter *et al.* unpublished), suggesting in this last study the occurrence of a mixing of gene pools of different origins, mediated by the oceanographic currents reaching this area, which is consistent with our findings. A second genetic barrier was located at the Almeria-Oran front. This front is a thermohaline density front generated by the convergence between the inflow of Atlantic water through the Strait of Gibraltar and the Mediterranean water. The Almeria-Oran front has been shown to represent a barrier to gene flow in numerous species (Patarnello *et al.* 2007; Galarza *et al.* 2009; Sala-Bozano *et al.* 2009) but does not affect all species (Patarnello *et al.* 2007; Garcia-Merchan *et al.* unpublished). This front seems to act as a weak barrier to gene flow in *P. clavata*.

Besides those genetic breaks, the significant and positive correlation between genetic differentiation and geographic distance suggested that distance is also a cause of barrier to gene flow in *P. clavata*. The regional pattern of isolation by distance underlined a short effective larval dispersal in this species, which is in agreement with the negative phototaxis behaviour of the larva, its short swimming period (Linares *et al.* 2008b) and its lecithotrophic character. A similar pattern of isolation by distance, with low larval dispersal ability, has already been reported for other Mediterranean sessile invertebrates (Duran *et al.* 2004; Blanquer & Uriz 2010; Ledoux *et al.* 2010b but see Costantini *et al.* 2007b).

At regional scale, the three regions studied in *P. clavata* displayed a pattern of IBD. A similar pattern was observed for *C. rubrum* in the Medes and Marseille regions, whereas this species did not exhibit this pattern in North Corsica (Ledoux *et al.* 2010b). In *P. clavata*, gene flow seemed to occur mainly in the direction of the dominant surface current: southwards in Medes and northwards in North Corsica. In the Gulf of Lion, a distinction between samples from Marseille and the MOU sample from Côte Bleue was observed. The genetic distinction between the two sides of the Marseille area had already been observed by Lejeusne & Chevaldonné (2006) in a cave-dwelling mysid, and the authors related it to a local hydrodynamic barrier. This barrier could be effective for *P. clavata,* and the high admixture found for PHN sample and the identification of a barrier at the regional scale might indicate a transition zone at this location. Nevertheless, this barrier was not observed for the red coral *C. rubrum* (Ledoux *et al.* 2010b). In Marseille, the distinction between the two main groups was not outright, supporting the occurrence of stochastic gene flow in this region with the influence of populations from Port-Cros that belong to a marine protected area. This result could explain the weak correlation found between genetic distance and geographic distance within this region. The summer circulation in the Gulf of Lion displays high spatiotemporal variability induced by different wind systems (Millot 1979), which may explain the stochastic pattern of genetic structure found in Marseille.

At local scale, the pattern of genetic structure between populations dwelling at different depths within the same location depended on the depth ranges. In Pota del Llop (15 and 35 m), Grotte Pérès and Petit Congloué (10 and 20 m), the results at the individual and population levels suggest the occurrence of gene flow between these depth ranges within the same location. In contrast, in Pharillons and Riou Sud

(20 and 40 m), the results/suggest restricted gene flow between 20 and 40 m. Genetic differentiation over similar depth ranges within a single location has been recently emphasized in the coral reef species *Seriatopora hystrix*, although vertical migration was observed in north-west but not in north-east Australian reefs (Van Oppen *et al.* 2011). These differences in the patterns of gene flow over depths between reefs have been related to differences in coral bleaching-related mortality (Van Oppen *et al.* 2011). In our study, such a phenomenon is unlikely to have occurred as our sampled locations did not recently undergo severe disturbances and first-generation migrants were not detected at the locations where gene flow was observed between depths. Other factors can explain our contrasted patterns, such as local topography, water flow and the occurrence of the summer thermocline. The confirmation of this last hypothesis could be significant to explain differences at local scale for *P. clavata* and other benthic species with similar reproductive modes. In general, summer conditions are characterized by the presence of a thermocline located at approximately 20 m depth in the NW Mediterranean region (Bensoussan *et al.* 2010). The thermocline may act as a physical barrier to gene flow leading to genetic differentiation between populations dwelling on either side of the thermocline. In Medes, the thermocline is deeper than in Marseille (reaching 40 m depth) because of recurrent downwellings (Bensoussan *et al.* 2010), which may explain the occurrence of gene flow in Medes between 15 and 35 m. The contrasted thermal regimes and the reduced gene flow between depths on either side of the thermocline may be a favourable factor for the evolution of local adaptation to temperature and depth. The occurrence of local adaptation within marine populations at small spatial scales has recently been reported in several species and may be more common than previously thought (Sanford & Kelly 2011). Adaptation to local environment over a 30 m depth range has been suggested as a cause of genetic structure across habitats in *Seriatopora hystrix* (Bongaerts *et al.* 2010). Local adaptation to temperature within this depth range also seems likely for *Corallium rubrum* (Torrents *et al.* 2008), and genotype–environment interactions related to depth have been observed in common garden experiments in this species in one of the locations (Riou, Marseille) also studied in the present work (Ledoux 2010). Taking into account the observed genetic differentiation related to depth in that locality in two gorgonian species, common environmental factors leading to local adaptation could be shaping genetic variability associated with depth in that region. Nevertheless, further locations and samples from both sides of the thermocline as well as common garden experiments are required to study these hypotheses.

Thus, limited larval dispersal in combination with hydrodynamic conditions may strongly influence the genetic structure of sessile marine species at different spatial scales. The study at different scales is thus important to uncover the factors shaping the genetic patterns of the species and should be considered in future studies. Despite the strong genetic structure in *P. clavata*, some gene flow might be occurring over short and large distances (mean of 160 km), which may be plausible given the potential duration of larval phase under experimental conditions (Linares *et al.* 2008b). However, the low percentage of migrants (0–14% per population) suggests that migration events among populations are sporadic. Consequently, our results suggest that *P. clavata* populations mainly rely on self-recruitment (between 86% and 100%, Table S3, Supporting Information).

Consequences and implications for the conservation of Paramuricea clavata

In the current context of global warming (IPCC 2007) and given the observed pattern of stratification enhancement owing to global warming (Coma *et al.* 2009) and the predictions of increased frequency of heat waves in the Mediterranean Sea (Diffenbaugh *et al.* 2007), our results regarding the evaluation of genetic diversity and population connectivity of *P. clavata* are of major importance to provide an assessment of population genetic data and a baseline for further genetic monitoring (Schwartz *et al.* 2007). Besides climatic threats, high diving activities have been shown to cause serious damages to red gorgonian populations by increasing their natural mortality rate (Coma *et al.* 2004). Simulations predicted that the combined effects of diving activities and the actual frequency of mass mortality events could lead populations to extinction within the near future (Linares & Doak 2010). The recovery of populations that have been affected by strong disturbances appears to be very slow (Cerrano *et al.* 2005; Linares *et al.* 2005, 2008a; Cupido *et al.* 2008; Linares & Doak 2010) because of the low dynamics of *P. clavata* populations (Coma *et al.* 1998, 2004; Linares *et al.* 2008a) and the low recruitment rates within populations (Coma *et al.* 2001). The high degree of population differentiation highlighted in this study suggests a limited effective dispersal, which implies that the recovery of populations will mainly rely on self-recruitment. However, at regional scale, sporadic gene flow may occur among *P. clavata* populations, most probably depending on hydrodynamic conditions. Thus, conservation plans for the Mediterranean red gorgonian should be defined at regional and local scales. Networks of marine reserves have been considered as a

key conservation strategy to protect biodiversity (Jones *et al.* 2007). To achieve this aim of protection, the design of a marine reserve network should take into consideration (i) connectivity among populations as it will ensure population persistence, (ii) genetic diversity as it could enhance population resilience in the face of disturbances (Almany *et al.* 2009) and (iii) control of diving activities within reserves to limit the effects of the more controllable forms of human-induced impact.

An experimental and modelling approach for restoration has been undertaken on red gorgonian populations and has suggested the feasibility of such a strategy to help populations to recover after disturbances at local scale (Linares *et al.* 2008d). Our genetic survey may also help such restoration projects because genetic factors (Baums 2008), such as the genotypic diversity of transplanted individual or putative adaptation of the individuals to their environment of origin, should be considered to avoid loss of fitness in restored populations (Williams 2001; Baums 2008).

Paramuricea clavata is a structuring key species, which provides biogenic substrate, shade and shelter for other species of the associated assemblages (True 1970; Ballesteros 2006) that may thus indirectly suffer from the damages caused to red gorgonians (Ribes & Coma 2005). Therefore, our study and the resulting guidelines for the conservation of the red gorgonian will enable the conservation of one of the most biodiversity-rich communities in the Mediterranean Sea.

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This article is part of the PhD work of K.M.-J., which focuses on the application of genetic approaches to study the population structure, mating system and dispersal in *Paramuricea clavata* for conservation purposes. M.P. is an associate professor whose research focuses on molecular population studies of marine and model organisms. J.-B.L. is a post-doctoral researcher interested in the evolution and conservation of coastal marine invertebrates. R.C. is a researcher interested in understanding the structure and functioning of marine ecosystems. J.G. is a researcher involved in marine population and community studies focused on the conservation of long-lived invertebrates. J.-P.F. is a researcher interested in the ecology and evolution of marine biodiversity. D.A. is an associate professor interested in population genetics and adaptive processes.

Data accessibility

Microsatellite data set: DRYAD entry doi:10.5061/dryad.s01rg.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 f estimator of F_{IS} per sample and locus and null allele frequencies.

Table S2 Pairwise F_{ST} (upper right) and D_{EST} (lower left) values.

Table S3 Individuals assigned as first-generation migrants as estimated by GENECLASS2.

Fig. S1 Correlation between genetic distances computed as $F_{ST}/(1-F_{ST})$ and the logarithm of geographical distances (m) within the three regions: (a) Medes, (b) Marseille and (c) North Corsica.

Fig. S2 Plot of LnP(D) as a function of the number of clusters (K) across the 10 runs: (a) for the whole data set (first round) and (b) for each of the five clusters (second round).

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